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# Interleukin-10 secretion from CD14<sup>+</sup> peripheral blood mononuclear cells is downregulated in patients with acne vulgaris

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## Summary

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The authors state no conflict of interest.

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**Background** Acne is a common chronic inflammatory dermatosis of the pilosebaceous unit. It is characterized by seborrhoea, comedone formation and an inflammatory response consistent with defective cellular immunity to *Propionibacterium acnes*.

**Objectives** The objective of this study was to investigate the immune reactivity of patients with acne compared with healthy controls by examining the response of peripheral blood mononuclear cells (PBMCs) to stimulation with *P. acnes*. Particular focus was placed upon measuring the production of interleukin (IL)-10, which has an established immunoregulatory role.

**Patients and methods** Venous blood was collected from 47 patients with acne and 40 age- and sex-matched healthy controls with no prior history of acne. PBMCs were cultured and their cytokine response to *P. acnes* investigated.

**Results** Pro-inflammatory IL-8 and tumour necrosis factor (TNF)- $\alpha$  secretion from PBMCs was higher in patients with acne when stimulated with *P. acnes*. In contrast, a statistically significant reduction in PBMC secretion of anti-inflammatory IL-10 in patients with acne was identified. The impaired production of IL-10 by PBMCs from patients with acne was confined to CD14<sup>+</sup> cells presumed to be monocytes. The ability of CD14 cells from patients with acne to phagocytose *P. acnes* bacteria was also observed to be defective but the addition of exogenous IL-10 to PBMC cultures restored phagocytic activity.

**Conclusions** These data suggest that patients with acne have a pro-inflammatory cytokine milieu and crucially are unable to contain early inflammatory changes due to a specific defect in immunosurveillance, namely low monocyte IL-10 production. Our observations raise the possibility that acne therapeutics might profitably target IL-10 both as a regulator of pro-inflammatory cytokines and in augmenting the CD14<sup>+</sup> cell phagocytic response.

Acne vulgaris is a very common dermatosis<sup>1</sup> characterized by an inflammatory reaction in the pilosebaceous follicles of the face and trunk. It is associated with androgen-mediated seborrhoea, ductal hypercornification and the production of inflammatory mediators by *Propionibacterium acnes* and/or *P. granulosum* trapped within the duct by a cornified plug.<sup>2</sup> The cellular infiltrate around early inflamed lesions and also healthy-looking follicles in acne-prone skin comprises predominantly CLA<sup>+</sup> T-helper (Th) 1 cells and CD68<sup>+</sup> macrophages.<sup>3</sup> Keratinocyte-derived interleukin (IL)-1 $\alpha$  is a key early mediator of

inflammation and comedogenesis.<sup>4</sup> However, *P. acnes* appears capable of inducing the production of a variety of pro-inflammatory cytokines such as IL-8, tumour necrosis factor (TNF)- $\alpha$ , granulocyte-monocyte colony-stimulating factor and IL-12p40 by peripheral blood mononuclear cells (PBMCs)<sup>5,6</sup> and/or keratinocytes.<sup>7</sup>

These findings have led to the perception that acne is associated with the dysregulation of the pro-inflammatory response to *P. acnes*. One potential counter-regulatory cytokine is IL-10, which profoundly inhibits many macrophage and

dendritic cell functions by downregulating antigen presentation as well as the production of cytokines, chemokines, nitric oxide, reactive oxygen species and co-stimulatory molecules.<sup>8,9</sup> Specifically, IL-10 is a strong inhibitor of IL-12 production in the skin and likely acts to contain excessive dermal inflammation.<sup>10</sup> Consequently, there is considerable interest in the role of IL-10 in a number of skin disorders both as a potential mediator of pathological change and as a therapeutic agent.<sup>11</sup>

The main cellular sources of IL-10 include CD14<sup>+</sup> macrophages, as well as Th2 and T-regulatory (T<sub>reg</sub>) lymphocytes, which mediate peripheral tolerance.<sup>12,13</sup> IL-10 levels increase in acne comedones after ultraviolet irradiation and induce a functional deficiency of antigen-presenting cells including Langerhans cells.<sup>14</sup> This may explain why sunlight has beneficial effects on acne in some patients. Although IL-10, as well as TNF- $\alpha$  and IL-8, is reported to be upregulated in established inflamed lesions,<sup>15</sup> its role in the control of early inflammatory events by newly recruited cells from the blood is not known.

Thus, there is a need for a greater understanding of the inflammatory response in acne and particularly the role of immunoregulatory IL-10. This study focused on the extent and cellular source of IL-10 secretion by PBMCs from patients with acne and healthy controls in response to *P. acnes*. The study demonstrates that IL-10 production by PBMCs, in particular CD14<sup>+</sup> cells, is significantly lower in patients with acne. This highlights a potentially important role for this cytokine in regulating the inflammatory response to *P. acnes* within follicles.

## Patients and methods

### Patients and controls

Individuals participating in this study were 47 patients with acne (27 males and 20 females with a mean age of 19, range 16–26 years) and 40 controls (15 males and 25 females with a mean age of 20, range 16–26 years). Patients were graded for their acne according to the Leeds scale<sup>16</sup> and were free of topical or systemic therapy for at least 2 weeks prior to blood collection. Ethical approval for this project was given by the Research Ethics Committee of Harrogate District Hospital (REC number: 05/Q1107/78). The study was conducted according to the principles of the Declaration of Helsinki and written informed consent was obtained from all participants.

### Bacterial culture

A single colony population of *P. acnes* (NCTC 737) was incubated at 37 °C for 2–3 days in Wilkins Chalgren anaerobic broth (Sigma-Aldrich Ltd, Poole, U.K.), washed three times, resuspended in NaCl (0.9%) and then frozen at –80 °C until further use. The viability of *P. acnes* after freezing was verified and it was determined that 100  $\mu$ g bacteria was equivalent to  $2 \times 10^6$  CFU.

### Isolation of peripheral blood mononuclear cells and *in vitro* culture

Venous blood (20 mL) was layered onto an equal volume of Histopaque (Sigma-Aldrich Ltd) and centrifuged for 30 min at 400 g. The PBMC interface layer was washed and then cultured at  $2 \times 10^5$  cells per well in RPMI 1640 supplemented with 10% low endotoxin fetal calf serum (Biosera, Ringmer, East Sussex, U.K.), 1% L-glutamine (200 nmol L<sup>-1</sup>) and 1% penicillin/streptomycin (10 000 U mL<sup>-1</sup>) (Gibco, Paisley, U.K.). Cells were stimulated with *P. acnes* (0.1–100  $\mu$ g mL<sup>-1</sup>), anti-CD3 monoclonal antibody (mAb) (0.5  $\mu$ g mL<sup>-1</sup>; R & D Systems, Abingdon, U.K.), or lipopolysaccharide (LPS) (from *Escherichia coli* 0111 B4, 500 ng mL<sup>-1</sup>, Sigma-Aldrich Ltd) at 37 °C and 5% CO<sub>2</sub>. The lowest concentrations of *P. acnes* (0.1 and 1.0  $\mu$ g mL<sup>-1</sup>) were tested on only a limited number (~16–20) of subjects, hence the variable number of patients given for different bacterial doses. PBMCs were cultured with live *P. acnes* without antibiotics.

### Analysis of peripheral blood mononuclear cell proliferation and cytokine detection

Cell supernatants were collected at 24 and 72 h, and following storage at –20 °C, were analysed for the presence of IL-8, IL-10, IL-12/23p40 and TNF- $\alpha$  using enzyme-linked immunosorbent assay (ELISA) detection kits (IL-8 kit from Biosource Europe SA, Nivelles, Belgium; others from R & D Systems). After 72 h culture, cells were incubated for a further 18 h in the presence of [<sup>3</sup>H]-thymidine at 0.2  $\mu$ Ci per well (Amersham plc, Amersham, Bucks, U.K.). Cell cultures were then harvested using a Filtermate Harvester (Packard Bioscience BV, Groningen, the Netherlands) and the incorporation of radioisotope into cellular DNA determined using a TopCount NXT<sup>TM</sup> scintillation counter (Packard Bioscience BV) and expressed as mean stimulation index ( $\pm$  SEM) of c.p.m. for stimulated cells over unstimulated cells for each control and patient.

### Intracellular interleukin-10 staining and analysis by flow cytometry

PBMCs were cultured for 48 h, the last 5 h of which were in the presence of 0.5  $\mu$ L per well Golgi Stop (BD Pharmingen, Oxford, U.K.). Cells were then washed and stained with fluorochrome-conjugated antibodies [diluted in phosphate-buffered saline (PBS) containing 10% bovine serum albumin] for 30 min at 4 °C. Antibodies were as follows: Pacific Blue-anti-human CD4 (clone OKT4), fluorescein-isothiocyanate-anti-CD19 (clone HIB19), biotin-anti-CD14 (clone 61D3; all BD Pharmingen) followed by PE-Cy7-labelled streptavidin (eBioscience, Hatfield, U.K.), or the appropriate labelled isotype control antibodies. Cells were then treated with Cytofix/Cytoperm kit (BD Pharmingen) and stained with Alexa Fluor 647-anti-human IL-10 mAb (clone JES3-9D7) or the corresponding isotype control, for 30 min at 4 °C. Cells were

analysed using a Cyan II flow cytometer (DakoCytomation, Ely, U.K.) with Summit<sup>TM</sup> software.

### Phagocytosis of *Propionibacterium acnes* in vitro by peripheral blood mononuclear cells

*P. acnes* cells were stained using Alexa Fluor 488 TFP (Alexa Fluor<sup>®</sup> 488; Invitrogen, Paisley, U.K.) for 30 min in the dark at 37 °C. Following 1 h incubation in cold PBS in the dark to remove unbound dye, labelled *P. acnes* were added to PBMCs for 45 min in the dark. Cells were then labelled with anti-CD14 antibody, and phagocytosis determined as the number of CD14<sup>+</sup> cells also positive for Alexa Fluor 488. In some experiments, PBMCs were pre-incubated with recombinant human IL-10 (10 ng mL<sup>-1</sup>; R & D Systems) for 2 h prior to the addition of *P. acnes* at 10 or 100 µg mL<sup>-1</sup>. Cell culture supernatants were analysed for IL-8, IL-12p40 and TNF-α by ELISA as above after 24 h stimulation.

### Statistical analysis

Statistical comparison between patient and control samples for each experiment was made using a Mann-Whitney U-test. Values of  $P < 0.05$  were considered significant.

## Results

### Levels of interleukin-12, tumour necrosis factor-α and interleukin-8 are similar, or elevated in patients with acne

In healthy controls and patients with acne, the secretion of pro-inflammatory IL-12p40 by PBMCs was actively stimulated by increasing concentrations of *P. acnes* with a peak response at 10 µg mL<sup>-1</sup> (492 ± 68 pg mL<sup>-1</sup>, Fig. 1a). However, there was no significant difference in the secretion profile between patients and controls. There was also little difference in the secretion of TNF-α in response to either LPS or low concentrations of *P. acnes* between patients with acne and healthy controls, although when stimulated with 10 or 100 µg mL<sup>-1</sup> *P. acnes* the level of TNF-α detected was significantly higher for PBMCs from patients with acne (both  $P < 0.05$ ). In response to *P. acnes*, IL-8 secretion was produced in a dose-dependent manner (Fig. 1c). Moreover, PBMCs from patients with acne in the presence of LPS or *P. acnes* (at 1 µg mL<sup>-1</sup> and 10 µg mL<sup>-1</sup>) secreted significantly more IL-8 than PBMCs from healthy controls ( $P < 0.05$ ).

### Secretion of the downregulatory cytokine interleukin-10 by peripheral blood mononuclear cells in response to *Propionibacterium acnes* is significantly lower in patients with acne

The peak of IL-10 production was detected at 72 h (data not shown) and secretion in response to *P. acnes* increased in a dose-dependent manner and reached a plateau at 10 µg mL<sup>-1</sup>

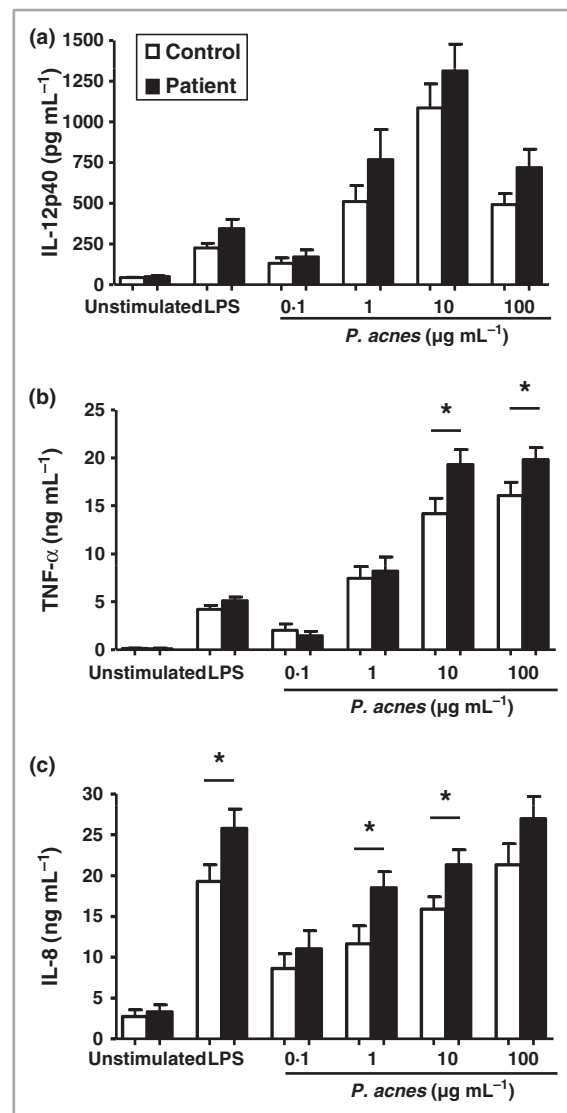
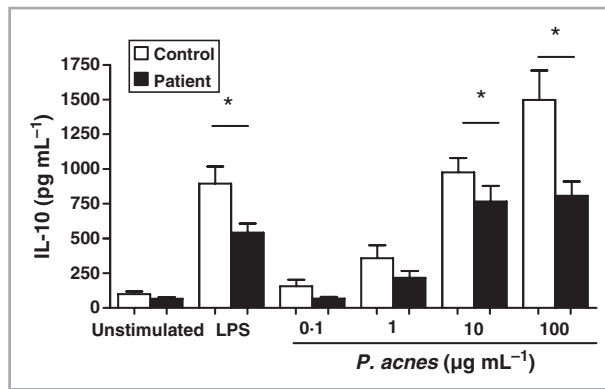


Fig 1. Peripheral blood mononuclear cells (PBMCs) from patients with acne secrete similar or greater amounts of pro-inflammatory cytokines than PBMCs from controls. (a) The secretion of interleukin (IL)-12p40 in patients with acne ( $n = 26-41$ ) and controls ( $n = 16-36$ ); (b) tumour necrosis factor (TNF)-α (patients  $n = 20-28$ ; controls,  $n = 12-25$ ); and (c) IL-8 (patients,  $n = 21-32$ ; controls,  $n = 11-28$ ) was measured by enzyme-linked immunosorbent assay where  $n$  values are greatest for *P. acnes* concentration of 10 and 100 µg mL<sup>-1</sup> and lowest for 0.1 and 1 µg mL<sup>-1</sup>. Results are expressed as the mean ± SEM. \* $P \leq 0.05$ .

in patients with acne and a maximum at 100 µg mL<sup>-1</sup> in controls (Fig. 2a). However, IL-10 secretion was significantly lower in patients with acne compared with controls (e.g. at 10 µg mL<sup>-1</sup>, 976 ± 102 cf. 766 ± 112 pg mL<sup>-1</sup>; and at 100 µg mL<sup>-1</sup>, 1498 ± 210 cf. 807 ± 103 pg mL<sup>-1</sup> for patients with acne and healthy controls, respectively; both  $P < 0.05$ ). Indeed, the level of the IL-10 response was on average 46% lower in patients with acne compared with controls for stimulation with *P. acnes* at 100 µg mL<sup>-1</sup>. Similarly, significantly less IL-10 was also secreted in response to LPS in



**Fig 2.** Interleukin (IL)-10 secretion by peripheral blood mononuclear cells (PBMCs) is significantly lower in patients with acne than in healthy controls. PBMCs incubated for 72 h in the absence or presence of lipopolysaccharides (LPS) or *P. acnes* (0.1, 1, 10 or 100 µg mL<sup>-1</sup>). IL-10 levels were detected by enzyme-linked immunosorbent assay. Results are expressed as the mean ± SEM (*n* = 20–40 for controls, *n* = 25–39 for patients). \**P* ≤ 0.05.

patients with acne when compared with the control PBMC response (541 ± 66 cf. 895 ± 122 pg mL<sup>-1</sup>; *P* < 0.05). No sex- or age-related difference in the production of IL-10 was demonstrated (data not shown).

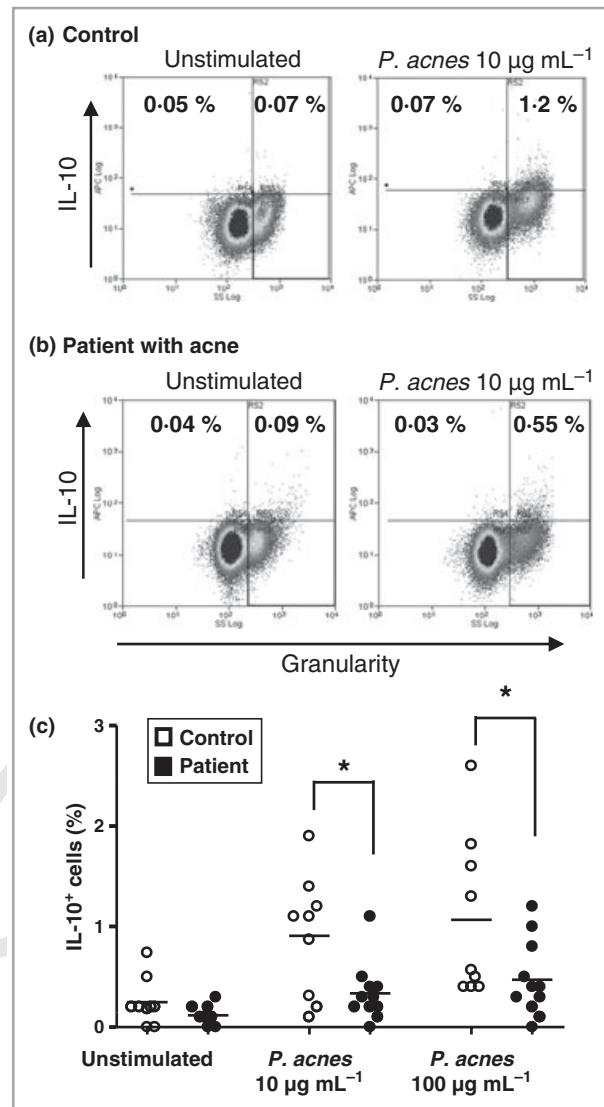
In order to identify a possible correlation between acne disease severity and IL-10 secretion in response to *P. acnes*, patient PBMCs were divided into two groups according to the severity of acne: mild acne with a grade ≤ 1, and moderate to severe acne with a grade > 1 (scored according to the scale of Burke and Cunliffe<sup>16</sup>). No difference in the level of IL-10 secretion was observed between patients with mild acne (*n* = 19) and those with moderate to severe acne (*n* = 16) (data not shown).

### Interleukin-10<sup>+</sup> peripheral blood mononuclear cells are less abundant in patients with acne

In resting healthy PBMCs, IL-10 was not detected, or at only a very low level (~0.2% Fig. 3a, c). However, when stimulated with 10 µg mL<sup>-1</sup> of *P. acnes*, the detection of IL-10<sup>+</sup> PBMCs was increased four-fold such that ~1.0% of the total PBMC population was positive for this cytokine. Although these values appear low, over 50 000 events were analysed by flow cytometry giving confidence that the observed changes are significant. The percentage of IL-10<sup>+</sup> PBMCs in patients with acne was low (0.3%) after stimulation with 10 µg mL<sup>-1</sup> *P. acnes*, and was significantly lower when compared with PBMCs from healthy controls (*P* < 0.05; Fig. 3b, c). Similar results were obtained using a higher stimulation dose of 100 µg mL<sup>-1</sup> *P. acnes* (*P* < 0.05). Overall, there were about 60% fewer IL-10<sup>+</sup> PBMCs from patients with acne than healthy controls (Fig. 3c).

### Proliferation of peripheral blood mononuclear cells from patients with acne and healthy controls is similar

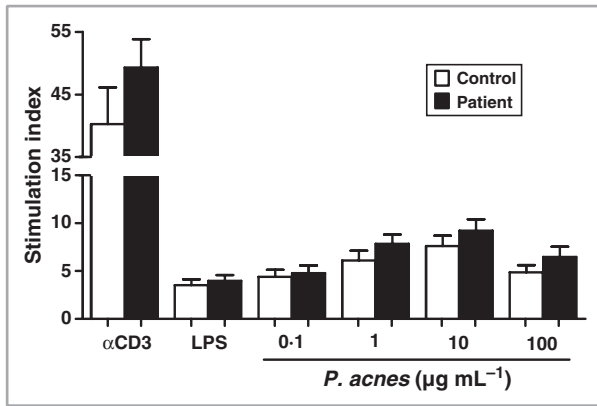
The proliferative response of PBMCs to *P. acnes* was evaluated in order to determine whether the difference in IL-10 production



**Fig 3.** Interleukin (IL)-10<sup>+</sup> cells are less abundant in patients with acne than in healthy controls. Intracellular IL-10 in peripheral blood mononuclear cells (PBMCs) from (a) a healthy control, and (b) from a patient with acne, determined following 48 h incubation with or without *P. acnes*. The percentage of IL-10<sup>+</sup> cells (minus the isotype control) is indicated. (c) IL-10<sup>+</sup> cells were significantly less abundant in PBMCs from patients with acne (*n* = 11) than healthy controls (*n* = 9) stimulated with *P. acnes*. \**P* ≤ 0.05.

was a reflection of PBMC proliferation. *P. acnes* induced the proliferation of PBMCs from healthy controls and patients with acne to a similar extent, with mean stimulation indices, respectively, of 7.6 ± 1.1 and 9.2 ± 1.2 over unstimulated cells for a dose of 10 µg mL<sup>-1</sup> but they were not statistically different (all *P* > 0.05; Fig. 4). In comparison, anti-CD3 mAb induced far higher levels of cell proliferation leading to stimulation indices of 65.1 ± 8.7 and 77.7 ± 7.2 for control and patient PBMCs, respectively, but again they were not significantly different.

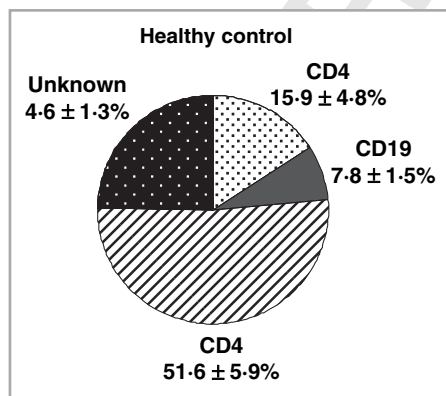




**Fig 4.** Proliferation of peripheral blood mononuclear cells (PBMCs) from patients with acne and healthy controls in response to mitogen and *P. acnes* stimulation. PBMCs were cultured for 72 h in the presence or absence of  $\alpha$ CD3 monoclonal antibody, lipopolysaccharide (LPS) or *P. acnes* (0.1, 1, 10 and 100  $\mu$ g mL<sup>-1</sup>). The incorporation of [<sup>3</sup>H] thymidine was measured in c.p.m. Results are expressed as the mean  $\pm$  SEM ( $n = 20$ –40 for controls,  $n = 19$ –33 for patients). \* $P \leq 0.05$ .

#### Patients with acne have significantly fewer interleukin-10<sup>+</sup> CD14<sup>+</sup> peripheral blood mononuclear cells

In healthy controls, the vast majority of IL-10<sup>+</sup> PBMCs were CD14<sup>+</sup> (51.6  $\pm$  9%). The percentage of IL-10<sup>+</sup> PBMCs that were CD4<sup>+</sup> or CD19<sup>+</sup> remained small, even after stimulation with *P. acnes* (15.9  $\pm$  4.8% and 7.8  $\pm$  1.5%, respectively; Fig. 5). The remaining 25% were CD14<sup>-</sup> CD4<sup>-</sup> CD19<sup>-</sup>, and possibly represent inactivated B cells, CD8<sup>+</sup> cells, NK cells or CD14<sup>-</sup> macrophages. Examination of the CD14<sup>+</sup> PBMC population from healthy individuals showed that when stimulated with 10  $\mu$ g mL<sup>-1</sup> *P. acnes*, 5.05  $\pm$  0.83% were IL-10<sup>+</sup>



**Fig 5.** The majority of interleukin (IL)-10<sup>+</sup> cells are CD14<sup>+</sup>. Relative contribution of CD4<sup>+</sup>, CD19<sup>+</sup> and CD14<sup>+</sup> cells in the detection of IL-10 in healthy controls peripheral blood mononuclear cells (PBMCs) treated with *P. acnes* (10  $\mu$ g mL<sup>-1</sup>). CD14<sup>+</sup> cells are the major source of IL-10. Similar proportions were observed for patients with acne PBMCs.

**Table 1** The percentage of IL-10<sup>+</sup> CD14<sup>+</sup> cells cultured in the absence or presence of *P. acnes* is lower in patients with acne

	CD14 <sup>+</sup>	CD19 <sup>+</sup>	CD4 <sup>+</sup>
Medium only			
Controls <sup>a</sup>	0.92 $\pm$ 0.25	0.54 $\pm$ 0.10	0.31 $\pm$ 0.08
Patients <sup>a</sup>	1.10 $\pm$ 0.17	0.32 $\pm$ 0.08	0.28 $\pm$ 0.08
<i>P. acnes</i> (10 $\mu$ g mL <sup>-1</sup> )			
Controls	<b>5.05 <math>\pm</math> 0.83</b>	0.61 $\pm$ 0.14	0.39 $\pm$ 0.13
Patients	<b>2.07 <math>\pm</math> 0.77*</b>	0.65 $\pm$ 0.15	0.33 $\pm$ 0.09
<i>P. acnes</i> (100 $\mu$ g mL <sup>-1</sup> )			
Controls	4.36 $\pm$ 0.72	0.68 $\pm$ 0.17	0.19 $\pm$ 0.04
Patients	3.09 $\pm$ 0.57	0.53 $\pm$ 0.19	0.36 $\pm$ 0.12

<sup>a</sup> $n = 8$ ; \* $P < 0.05$  for patients compared with controls; bold, statistically significant.

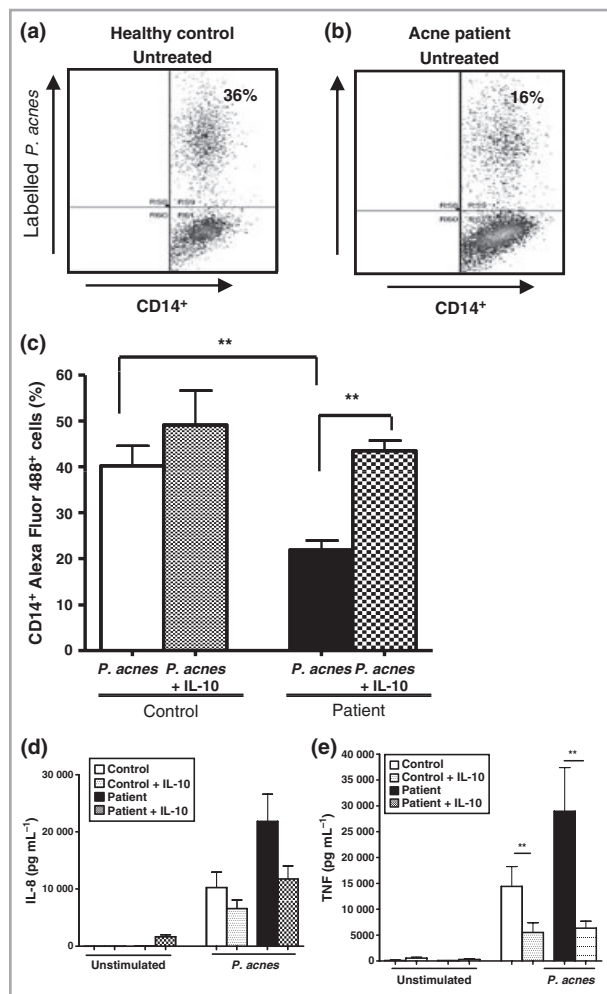
(Table 1). This is significantly higher than in patients with acne where only 2.07  $\pm$  0.77% were IL-10<sup>+</sup> ( $P < 0.05$ ). However, no significant change between patients with acne and healthy controls in the proportion of IL-10<sup>+</sup> PBMCs were observed for CD19<sup>+</sup> or CD4<sup>+</sup> populations (both  $P > 0.05$ ). This suggests that the deficiency in IL-10 production in patients with acne is confined to the CD14<sup>+</sup> cell population and is not evident in CD4<sup>+</sup> T or CD19<sup>+</sup> B lymphocytes.

#### The phagocytic activity of CD14<sup>+</sup> peripheral blood mononuclear cells is significantly reduced in patients with acne, but is restored by the addition of exogenous interleukin-10

The phagocytic activity of CD14<sup>+</sup> PBMCs was examined in light of their ability to take up Alexa Fluor 488 labelled *P. acnes*. It was observed that CD14<sup>+</sup> cells from patients with acne phagocytosed significantly fewer labelled *P. acnes* than cells from healthy controls (i.e. ~20% cf. 40%) (Fig. 6a–c,  $P < 0.01$ ). Furthermore, the addition of IL-10 to PBMC cultures increased the uptake of *P. acnes* by CD14<sup>+</sup> cells from patients with acne by approximately two-fold ( $P < 0.01$ ), to a level similar to that seen in healthy controls (Fig. 6a–c). Supplementation of IL-10 to cultures of PBMCs from healthy controls had no significant impact on their ability to phagocytose *P. acnes* ( $P > 0.05$ ). The addition of IL-10 to PBMCs from acne and healthy controls stimulated with *P. acnes* (10  $\mu$ g mL<sup>-1</sup>) also led to a reduced secretion of IL-8 (Fig. 6d) while the effect of IL-10 supplementation on TNF- $\alpha$  secretion by PBMCs stimulated with *P. acnes* (10  $\mu$ g mL<sup>-1</sup>) was more pronounced with a reduction of 78% and 62% for PBMCs from patients with acne and healthy controls, respectively (Fig. 6e).

#### Discussion

The aim of this study was to investigate the inflammatory cytokine response of PBMCs from patients with acne vulgaris following stimulation with *P. acnes*. The production of the pro-inflammatory cytokines IL-8, TNF- $\alpha$  and IL-12p40 were either



**Fig 6.** CD14<sup>+</sup> peripheral blood mononuclear cells (PBMCs) from patients with acne phagocytose less *P. acnes* than cells from healthy controls, and levels of phagocytosis are restored by the addition of exogenous interleukin (IL)-10. PBMCs from healthy controls and from patients with acne ( $n = 4$ ) were stimulated with Alexa Fluor 488 labelled *P. acnes* in the presence or absence of recombinant IL-10. The level of phagocytosis was determined by the uptake of labelled *P. acnes* by CD14<sup>+</sup> PBMCs from (a) a healthy control or (b) an acne patient as determined by flow cytometry. (c) The addition of IL-10 to PBMCs from patients with acne restored levels of phagocytosis of *P. acnes* to levels seen in healthy controls and reduced the production of (d) IL-8 and (e) TNF- $\alpha$ . Data were normalized by a log10 for statistical analysis ( $P$ -values:  $**P \leq 0.01$ ).

slightly elevated or unchanged in patients with acne when compared with healthy controls. However, a statistically significant reduction in PBMC secretion of anti-inflammatory IL-10 in patients with acne was identified. The impaired production of IL-10 by PBMCs from patients with acne was confined to CD14<sup>+</sup> cells that were also defective in their ability to phagocytose *P. acnes* bacteria. The addition of exogenous IL-10 to PBMC cultures from patients with acne restored phagocytic activity.

To our knowledge, this study is the first to report a significant imbalance in the production by PBMCs of pro- and

anti-inflammatory cytokines between patients with acne and healthy controls. The secretion of both IL-8 and TNF- $\alpha$  by PBMCs was significantly elevated in patients with acne in agreement with studies elsewhere.<sup>17</sup> The production of these cytokines in response to *P. acnes* stimulation is linked to the expression of innate immune response pattern recognition receptor TLR2.<sup>5,18,19</sup> However, no difference was detected in the secretion of the p40 subunit of the pro-inflammatory cytokine complex IL-12/IL-23. This conflicts with findings that IL-12 originating from monocytes was increased following stimulation with *P. acnes* via TLR2,<sup>5,19</sup> but agrees with the observation that IL-12p40 mRNA is not upregulated in acne lesions compared with uninvolved skin.<sup>15</sup>

In contrast to the upregulation of pro-inflammatory cytokines, the production of the anti-inflammatory cytokine IL-10 was markedly downregulated in patients with acne in this study. IL-10 is a well-described regulatory cytokine that acts to harness the release of several pro-inflammatory cytokines.<sup>20</sup> In our study, it appears to act primarily on TNF- $\alpha$  but has little effect upon IL-12. Therefore, we speculate that in patients with acne, early activation of the immune response is not controlled, due to the relative paucity of IL-10. As a result, inflammation is pathologically sustained in patients with acne contributing to the events that lead to the initiation and/or persistence of inflammatory lesions. On the other hand, there was no association between the severity of acne and the absolute quantity of secreted IL-10. This might suggest that a relative deficiency of IL-10 predisposes a patient to acne, but cannot be used to predict the severity of the clinical lesions. It may also suggest that a lack of IL-10 cannot solely predict the severity of acne. Indeed, it is well known that acne is multifactorial and it would be surprising if it could be completely explained through the actions of a solitary cytokine.<sup>21</sup>

IL-10 can derive from CD4<sup>+</sup> T cells with regulatory function (so-called T<sub>regs</sub>).<sup>12,22</sup> However,  $\alpha$ CD3 mAb stimulation did not affect the differential secretion of IL-10 between PBMCs derived from patients with acne and controls. We also show that CD4<sup>+</sup> cells are only a minor (~16%) source of IL-10 and the proportions of IL-10<sup>+</sup> CD4<sup>+</sup> cells do not differ between control and patient PBMCs. Therefore, we conclude that IL-10-producing T<sub>regs</sub> among the PBMC population are unlikely to have a major role in determining the propensity to develop acne.

The most abundant IL-10<sup>+</sup> cell population among PBMCs were CD14<sup>+</sup> (> 50%) and these are under-represented in patients with acne. CD14<sup>+</sup> PBMCs are a well-described source of IL-10.<sup>23</sup> In the skin, the main sources of IL-10 in the early phase of inflammation are CD14<sup>+</sup> cells,<sup>15</sup> possibly originating from the peripheral blood. TLR2 expression on CD14<sup>+</sup> cells is also increased in cells localized within acne lesions.<sup>5,24</sup> The role of IL-10 is likely the downregulation of pro-inflammatory cytokines as demonstrated by the decreased production of IL-8 and TNF- $\alpha$  after the addition of exogenous IL-10 to PBMC cultures. However, IL-10 is known to be markedly upregulated in established acne lesions.<sup>15</sup> This appears to contradict the findings presented here. We speculate that the CD14<sup>+</sup> PBMCs

represent a set of circulating monocytes/immature macrophages that will be the first to infiltrate the tissue around a developing lesion. Dysregulation in IL-10 production by these circulating CD14<sup>+</sup> cells may facilitate the development of intense inflammatory lesions driven by an imbalance towards pro-inflammatory cytokines such as TNF- $\alpha$  and IL-8. Therefore, a deficit in the number of IL-10-producing CD14<sup>+</sup> monocytes, as seen in our patients with acne, would favour the development of an inflammatory cascade characteristic of acne lesions. In established lesions, CD68<sup>+</sup> mature macrophages will predominate<sup>3</sup> and only then is IL-10 finally produced in an attempt to regulate the overactive inflammatory response.

The cause of the differences in the production of IL-10 between healthy controls and patients with acne is uncertain. There is known to be considerable inter-individual variation in the capacity of human PBMCs to produce IL-10, with at least 50% likely due to genetic factors.<sup>25</sup> Polymorphisms of the 5'-flanking region of the IL-10 gene<sup>26</sup> and single nucleotide polymorphisms within the promoter region have been linked with a number of chronic dermatoses including psoriasis.<sup>27,28</sup> Unfortunately, a detailed genetic analysis was beyond the scope of the current study but the familial inheritance of acne may be partly explained by this mechanism. Our study also raises questions about the onset and resolution of acne. Onset commonly occurs during adrenarche when output of dihydroepiandrosterone (DHEA) starts to rise.<sup>29</sup> The relationship between DHEA, IL-10 and progression to acne has not been investigated, although data in humans indicate there may be a negative correlation between serum IL-10 levels and DHEA concentration.<sup>30,31</sup> The resolution of acne in individuals may represent upregulation of IL-10 expression and it would be important to perform a longitudinal study to determine if the course of acne relates to changes of IL-10 with age. Alternatively, other mechanisms of immune regulation may evolve with time and act independently of IL-10 released by CD14<sup>+</sup> cells. For example, T<sub>reg</sub> cells may be involved in the resolution of acne<sup>21</sup> and have been implicated in other skin conditions,<sup>32,33</sup> while the delivery of T<sub>reg</sub> cells can dampen skin inflammation in a model of psoriasis.<sup>34</sup> Therefore, it is reasonable to assume that T<sub>reg</sub> cells may downregulate the CD4<sup>+</sup> T-cell response to *P. acnes* and thus explain why resolution can occur even in the presence of reduced IL-10 production by PBMCs.

Administration of exogenous IL-10 *in vivo* is reported to be an effective therapeutic strategy in controlling skin disorders such as psoriasis.<sup>35</sup> This study raises the possibility that the same treatment strategy may be a feasible approach in acne. Moreover, and perhaps counterintuitively in light of its down-regulatory functions, administration of IL-10 to *in vitro* cultures of PBMCs from patients with acne increased the phagocytic process allowing CD14<sup>+</sup> cells to take up more *P. acnes*. Thus, in addition to limiting the levels of pro-inflammatory cytokines and so preventing a 'hyper-immune response', as seen in patients with acne, IL-10 also acts to facilitate phagocytosis by CD14<sup>+</sup> cells<sup>36</sup> and presumably results in more effective clearance of *P. acnes*.

In summary, these data suggest that acne lesions may develop because of a defect in immunosurveillance. We propose that an immune response to *P. acnes* bacteria in the follicle is usually dampened by PBMC-derived IL-10 before visible inflammation develops. Reduced production of IL-10 by CD14<sup>+</sup> PBMCs, most likely monocytes, predisposes to unchecked pro-inflammatory changes. This interpretation raises the possibility that acne therapeutics might profitably target IL-10 both as a regulator of pro-inflammatory cytokines and in augmenting the CD14<sup>+</sup> cell phagocytic response. Future studies should seek to characterize the cellular sources of IL-10 in evolving and healing acne lesions and compare the skin distribution of IL-10 in subjects with acne and subjects whose acne has resolved spontaneously.

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## References

- 1 Stathakis V, Kilkenny M, Marks R. Descriptive epidemiology of acne vulgaris in the community. *Australas J Dermatol* 1997; **38**:115–23.
- 2 Eady EA, Cove JH. Is acne an infection of blocked pilosebaceous follicles? Implications for antimicrobial treatment. *Am J Clin Dermatol* 2000; **1**:201–9.
- 3 Jeremy AH, Holland DB, Roberts SG *et al.* Inflammatory events are involved in acne lesion initiation. *J Invest Dermatol* 2003; **121**:20–7.
- 4 Ingham E, Eady EA, Goodwin CE *et al.* Pro-inflammatory levels of interleukin-1 alpha-like bioactivity are present in the majority of open comedones in acne vulgaris. *J Invest Dermatol* 1992; **98**:895–901.
- 5 Kim J, Ochoa MT, Krutzyk SR *et al.* Activation of toll-like receptor 2 in acne triggers inflammatory cytokine responses. *J Immunol* 2002; **169**:1535–41.
- 6 Sugisaki H, Yamanaka K, Kakeda M *et al.* Increased interferon-gamma, interleukin-12p40 and IL-8 production in *Propionibacterium acnes*-treated peripheral blood mononuclear cells from patient with acne vulgaris: host response but not bacterial species is the determinant factor of the disease. *J Dermatol Sci* 2009; **55**:47–52.
- 7 Schaller M, Loewenstein M, Borelli C *et al.* Induction of a chemoattractive proinflammatory cytokine response after stimulation of keratinocytes with *Propionibacterium acnes* and coproporphyrin III. *Br J Dermatol* 2005; **153**:66–71.
- 8 Ma J, Chen T, Mandelin J *et al.* Regulation of macrophage activation. *Cell Mol Life Sci* 2003; **60**:2334–46.
- 9 Moore KW, de Waal Malefyt R, Coffman RL *et al.* Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19**:683–765.
- 10 Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003; **3**:133–46.
- 11 Weiss E, Mamelak AJ, La Morgia S *et al.* The role of interleukin 10 in the pathogenesis and potential treatment of skin diseases. *J Am Acad Dermatol* 2004; **50**:657–75.



- 12 Belkaid Y, Rouse BT. Natural regulatory T cells in infectious disease. *Nat Immunol* 2005; **6**:353–60.
- 13 Birch KE, Vukmanovic-Stejić M, Reed JR *et al.* The immunomodulatory effects of regulatory T cells: implications for immune regulation in the skin. *Br J Dermatol* 2005; **152**:409–17.
- 14 Suh DH, Kwon TE, Youn JI. Changes of comedonal cytokines and sebum secretion after UV irradiation in acne patients. *Eur J Dermatol* 2002; **12**:139–44.
- 15 Kang S, Cho S, Chung JH *et al.* Inflammation and extracellular matrix degradation mediated by activated transcription factors nuclear factor-kappaB and activator protein-1 in inflammatory acne lesions in vivo. *Am J Pathol* 2005; **166**:1691–9.
- 16 Burke BM, Cunliffe WJ. The assessment of acne vulgaris – the Leeds technique. *Br J Dermatol* 1984; **111**:83–92.
- 17 Basal E, Jain A, Kaushal GP. Antibody response to crude cell lysate of *Propionibacterium acnes* and induction of pro-inflammatory cytokines in patients with acne and normal healthy subjects. *J Microbiol* 2004; **42**:117–25.
- 18 Vowels BR, Yang S, Leyden JJ. Induction of proinflammatory cytokines by a soluble factor of *Propionibacterium acnes*: implications for chronic inflammatory acne. *Infect Immun* 1995; **63**:3158–65.
- 19 Liu PT, Phan J, Tang D *et al.* CD209(+) macrophages mediate host defense against *Propionibacterium acnes*. *J Immunol* 2008; **180**:4919–23.
- 20 Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; **180**:5771–7.
- 21 Wilcox HE, Farrar MD, Cunliffe WJ *et al.* Resolution of inflammatory acne vulgaris may involve regulation of CD4<sup>+</sup> T-cell responses to *Propionibacterium acnes*. *Br J Dermatol* 2007; **156**:460–5.
- 22 Dudda JC, Perdue N, Bachtanian E *et al.* Foxp3<sup>+</sup> regulatory T cells maintain immune homeostasis in the skin. *J Exp Med* 2008; **205**:1559–65.
- 23 Keller CC, Yamo O, Ouma C *et al.* Acquisition of hemozoin by monocytes down-regulates interleukin-12 p40 (IL-12p40) transcripts and circulating IL-12p70 through an IL-10-dependent mechanism: in vivo and in vitro findings in severe malarial anemia. *Infect Immun* 2006; **74**:5249–60.
- 24 Jugeau S, Tenaud I, Knol AC *et al.* Induction of toll-like receptors by *Propionibacterium acnes*. *Br J Dermatol* 2005; **153**:1105–13.
- 25 Reuss E, Fimmers R, Kruger A *et al.* Differential regulation of interleukin-10 production by genetic and environmental factors – a twin study. *Genes Immun* 2002; **3**:407–13.
- 26 Eskdale J, Kube D, Gallagher G. A second polymorphic dinucleotide repeat in the 5' flanking region of the human IL10 gene. *Immunogenetics* 1996; **45**:82–3.
- 27 Hensen P, Asadullah K, Windemuth C *et al.* Interleukin-10 promoter polymorphism IL10.G and familial early onset psoriasis. *Br J Dermatol* 2003; **149**:381–5.
- 28 Kingo K, Koks S, Silm H *et al.* IL-10 promoter polymorphisms influence disease severity and course in psoriasis. *Genes Immun* 2003; **4**:455–7.
- 29 Stewart ME, Downing DT, Cook JS *et al.* Sebaceous gland activity and serum dehydroepiandrosterone sulfate levels in boys and girls. *Arch Dermatol* 1992; **128**:1345–8.
- 30 Tabata N, Tagami H, Terui T. Dehydroepiandrosterone may be one of the regulators of cytokine production in atopic dermatitis. *Arch Dermatol Res* 1997; **289**:410–14.
- 31 Suzuki T, Suzuki N, Engleman EG *et al.* Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1995; **99**:251–5.
- 32 Werfel T, Wittmann M. Regulatory role of T lymphocytes in atopic dermatitis. *Chem Immunol Allergy* 2008; **94**:101–11.
- 33 Kuhn A, Beissert S, Krammer PH. CD4(+)CD25(+) regulatory T cells in human lupus erythematosus. *Arch Dermatol Res* 2009; **301**:71–81.
- 34 Teige I, Hvid H, Svensson L *et al.* Regulatory T cells control VEGF-dependent skin inflammation. *J Invest Dermatol* 2009; **129**:1437–45.
- 35 Friedrich M, Docke WD, Klein A *et al.* Immunomodulation by interleukin-10 therapy decreases the incidence of relapse and prolongs the relapse-free interval in psoriasis. *J Invest Dermatol* 2002; **118**:672–7.
- 36 Lingnau M, Hofflich C, Volk HD *et al.* Interleukin-10 enhances the CD14-dependent phagocytosis of bacteria and apoptotic cells by human monocytes. *Hum Immunol* 2007; **68**:730–8.

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